A Phase 1 clinical trial of a DNA vaccine for Venezuelan equine encephalitis delivered by intramuscular or intradermal electroporation

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Abstract

Background

Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne alphavirus, which causes periodic epizootics in equines and is a recognized biological defense threat for humans. There are currently no licensed vaccines against VEEV available in the United States. We developed a candidate DNA vaccine expressing the E3-E2-6K-E1 genes of VEEV (pWRG/VEEV) and performed a Phase 1 clinical trial to assess safety, reactogenicity, tolerability, and immunogenicity.

Methods

Subjects were randomized into five groups and were vaccinated with high and low doses of pWRG/VEE or a saline placebo by intramuscular (IM) or intradermal (ID) electroporation (EP) using the Ichor Medical Systems TriGrid^{TM} Delivery System. Subjects in IM-EP groups received 0.5 mg (N=8) or 2.0 mg (N=9) of pWRG/VEE or saline (N=4) in a 1.0 ml injection. Subjects in ID-EP groups received 0.08 mg (N=8) or 0.3 mg (N=8) of DNA or saline (N=4) in a 0.15 ml injection. Subjects were monitored for a total period of 360 days.

Results:

No vaccine- or device-related serious adverse events were reported. Based on the results of a subject questionnaire, the IM- and ID-EP procedures were both considered to be generally acceptable for prophylactic vaccine administration, with the acute tolerability of ID EP delivery judged to be greater than that of IM-EP delivery. All

subjects (100%) in the high and low dose IM-EP groups developed detectable VEEV-neutralizing antibodies after two or three administrations of pWRG/VEE, respectively. VEEV-neutralizing antibody responses were detected in seven of eight subjects (87.5%) in the high dose and five of eight subjects (62.5%) in the low dose ID EP groups after three vaccine administrations. There was also a correlation between the DNA dose and the magnitude of the resulting VEEV-neutralizing antibody responses for both IM and ID EP delivery.

Conclusions

The candidate vaccine, pWRG/VEE delivered by either IM- or ID-EP is safe, tolerable, and immunogenic in humans at the evaluated dose levels.

Keywords: Venezuelan equine encephalitis, DNA vaccine, electroporation, intramuscular, intradermal, human, clinical trial

1. Introduction

Venezuelan equine encephalitis virus (VEEV) is an mosquito-borne alphavirus that causes periodic epizootics in the Americas, with infected equines serving as amplifying hosts [1]. Clinical symptoms of VEEV infections may include fever, headache, vomiting, malaise and, rarely, an encephalitic phase with somnolence [2]. Case-fatality rates for VEE are low (≤1%) [3]; however, VEEV is also recognized as a significant biological defense threat due to its ease of production, considerable stability, high infectivity through aerosol exposure, and low human infective dose (reviewed in [4]). As a result, VEEV has been classified as a Category B priority pathogen by both the Centers for Disease Control and Prevention and the National Institute of Allergy and Infectious Diseases.

To date, VEE vaccines have not been licensed for use in the U.S. Formalin-inactivated and live-attenuated vaccine candidates have used in humans under Investigational New Drug (IND) status, but the poor immunogenicity of the inactivated vaccine [5-7] and the reactogenicity of the live vaccine have prompted studies on alternative approaches, including DNA vaccines. DNA vaccines have shown promise in laboratory and early stage clinical studies for a variety of pathogens, and importantly for biodefense purposes, this platform has manufacturing and stability properties that are conducive to rapid production and efficient stockpiling [8].

We developed a candidate VEEV DNA vaccine candidate, pWRG/VEE, and have tested it in mice, rabbits and nonhuman primates (NHPs). When delivered by intramuscular (IM) electroporation (EP) to mice, pWRG/VEE elicited robust antibody responses, to include high levels of VEEV-neutralizing antibodies, in all three animal

species and provided protection against VEEV aerosol challenge in mice and NHPs [9]. The VEEV-neutralizing antibodies elicited in rabbits persisted at high levels for at least 6 months after vaccination.

Comparing IM- to ID-EP administration of pWRG/VEE in rabbits and NHPs demonstrated that both delivery methods elicited virus-neutralizing antibody responses of similar magnitudes. Moreover, similar levels of protection against aerosol VEEV challenge were elicited in NHPs receiving PWRG/VEE by IM- or ID-EP (Dupuy, et al., manuscript in preparation)**.** These encouraging results led us to conduct a Phase 1 study to assess and compare the safety, reactogenicity, tolerability, and immunogenicity of pWRG/VEE delivered at various doses by IM- or ID-EP.

2. Materials and methods

2.1. Vaccine and placebo

Construction of pWRG/VEE DNA vaccine candidate expressing the E3-E2-6K-E1 genes of VEEV subtype IAB was described previously [9]. The vaccine plasmid was produced under current Good Manufacturing Practices (Althea Technologies, Inc., San Diego, CA) and vialed at 2.0 mg/ml in phosphate buffered saline. Flow-cytometry-based *in vitro* potency assays using a VEEV E1-specific monoclonal antibody to detect expression in transfected cells were performed as described earlier [10]. Subjects received the vaccine as vialed (high dose), diluted 1:3 in 0.9% Sodium Chloride Injection, USP (Hospira, Inc. NDC 0409-4888-10) (low dose), or the diluent with no vaccine (placebo).

2.2. Electroporation delivery devices

The clinical use of the TDS-IM EP delivery device (Ichor Medical Systems, Inc., San Diego, CA) has been described [11]. For the TDS-ID device, the injectate was administered to the target tissue via needle-free jet injection (Medi-jector Vision, Antares Pharma) with distribution of the agent followed by the localized application of the EP inducing electrical fields with a 330 V/cm amplitude, 40 mS duration, and 10% duty cycle.

2.3. Overview of the clinical study design and enrollment of subjects

The study was sponsored by Ichor Medical Systems and conducted at Optimal Research (Accelovance), San Diego, CA, using a randomized, observer-blind, placebo-controlled, single-center design (IND # 015748). All recruiting and consent methods and materials were compliant with current Good Clinical Practice (GCP) guidelines and approved by the Aspire Institutional Review Board (IRB) http://aspire-irb.com and the Western IRB institutional biosafety committee was the Western IRB IBC (https://www.wirb.com/Pages/IBCServices.aspx). The Subjects were prescreened by plaque reduction neutralization tests (PRNT) for the absence of neutralizing antibodies to VEEV as described previously [12] and were then randomized to receive either three doses of pWRG/VEE or three doses of placebo at days 0, 28, and 56 administered by either ID-EP or IM-EP. Final pWRG/VEE doses were 0.5 or 2.0 mg of DNA delivered by IM-EP and 0.08 or 0.3 mg delivered by ID-EP (Fig. 1).

2.4. *Safety and immunogenicity*

Safety was assessed at each dose administration (days 0, 28, and 56), at follow up visits two and 14 days after each dosing, and at study weeks 20, 32, and 52. Measurement of vital signs, assessment of injection site reactions, and a review of systemic reactions were performed at each study visit. Subjects were provided a memory aid, oral thermometer, and measuring device to assist in the daily documentation of any symptoms/local reactions occurring within 14 days of each dosing. Adverse events (AEs) were assessed by the investigator for severity and potential relationship to the vaccine candidate and/or administration procedure and each event was graded as: Grade 1 (mild, does not interfere with routine activities); Grade 2 (moderate, interferes with routine activities); Grade 3 (severe, unable to perform routine activities); Grade 4 (hospitalization or ER visit for potentially life-threatening event). Blood and urine samples were obtained at each follow up visit. Safety labs included albumin, sodium, potassium, glucose, bilirubin, blood urea nitrogen (BUN), creatinine, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), complete blood count with differential, and urinalysis. Neutralizing antibodies against VEEV were measured for serum samples collected on days 0, 14, 42, 70, 140, 224, and 365 by PRNT as described previously [12].

2.5. Statistical methods

Descriptive analysis of safety and reactogenicity outcomes were performed for all subjects who received at least one dose of the DNA vaccine and for whom safety data were available. Summary tables were created in which incidence, intensity, and relationship to use of investigational product of individual solicited signs, symptoms, and

other events were delineated by study group, severity, gender, and overall. Unsolicited AEs and serious AEs (SAEs) were analyzed in a similar fashion. For hematology and serum chemistry tests, any clinically significant change from baseline value was identified. The median, inter-quartile range and normal values for each of the laboratory values (as determined by the contract laboratory) were reported for each treatment group for each specimen collection point.

For the immunogenicity evaluation, the primary analysis variable was the proportion of seropositive subjects ($PRNT_{80} \geq 1:10$) overall rate of seroconversion over all scheduled time points to study completion for each experimental group, the magnitude of the immunological response as well as the kinetics of the neutralizing antibody response and the duration of seropositivity. For each treatment group, a binomial proportion and exact 95% confidence interval (CI) were calculated. The secondary analysis variable is the geometric mean titers, with 95% CIs, of the $PRNT_{80}$ for VEEV-specific antibodies at each scheduled time point. Geometric mean titers, standard errors, and 95% CIs were calculated using log-transformed titers, replacing any titers below the limit of detection with 1.

3. **Results and Discussion**

3.1. Clinical subject population and conduct of the study

The planned enrollment for this Phase 1 study included five randomized 8 subject groups for a total of 40 subjects, each administered a total of 3 injections of PWRG/VEE or placebo at days 0, 28, and 56 (Figure 1). Vaccinations were performed at one of two pWRG/VEE DNA concentrations: 0.5 mg/ml or 2.0 mg/ml. Two groups of

subjects received administrations of the PWRG/VEE at the 0.5 mg/ml DNA concentration (“low dose”) by IM-EP (0.5 mg DNA dose) or ID-EP (0.08 mg DNA dose), and two groups were administered the PWRG/VEE at the 2.0 mg/ml concentration (“high dose”) by IM-EP (2.0 mg DNA dose) or ID-EP (0.3 mg dose) (Fig. 1). A placebo control group consisted of eight subjects, four of whom were given sterile saline by ID-EP (0.15 ml) or and four by IM-EP (1.0 ml). Intradermal injections of vaccine candidate or placebo were administered at a volume of 150 µl. Intramuscular injections of vaccine candidate or placebo was administered at a volume of 1000 µl. A total of 42 subjects were enrolled in the study, 41 of which received at least one administration and were evaluable for safety and immunogenicity. A subject in the high dose ID-EP group withdrew consent prior to the administration of the first dose and was replaced.

The 41 healthy adults comprising the study population ranged in age from 18 to 47 years (**Figure 1, Table 1**) with mean and median ages of enrolled subjects at 32.4 years and 35 years, respectively. The 41 subjects enrolled included 16 males and 25 females. Races enrolled included African American, white, Asian, native Hawaiian or other Pacific Islander, and other. The ethnicities were predominantly non-Hispanic/Latino (**Table 1**). One subject in the study population was assigned to the high dose IM-EP group and withdrew consent after the first administration due to non-AE related reasons and was replaced. The remaining 40 subjects in the study population received all vaccinations as scheduled and 38 subjects completed all study procedures (**Figure 1**). The two remaining subjects moved from the study area during the follow up period after all administrations were performed. One of these was in the placebo control

group and discontinued participation after the Day 70 visit and the other was in the high dose ID-EP group and discontinued participation after the day 140 visit.

3.3. Safety assessment

All subjects who received at least one dose of vaccine were evaluated for safety throughout the course of the study. In general, administration of pWRG/VEE by IM-EP or ID-EP was well tolerated. A total of 674 AEs were recorded for all subjects over the course of the study (Table 2). The vast majority of the AEs (512 of the 674; 75.6%) were classified as injection site reactions. These included injection site pain, erythema, induration, swelling, tenderness, bruising, punctures at the site of needle penetration, eschar formation at the site of needle penetration, and/or localized pigmentation changes. A total of 484 of the 512 (94.5%) injection site events were judged to be Grade 0 or 1 severity. Twenty six of the 512 (5%) injection site reactions were judged to be Grade 2 severity. Two of the 512 (0.3%) injection site reactions (both were local injection site pain) were judged to be Grade 3. However, both resolved within 24 hours after administration.

Although the overall frequency of injection site reactions was comparable between the two routes of administration, there were some differences in the types of reactions that were observed. Specifically, the subjects receiving ID-EP vaccine delivery exhibited higher rates of erythema, induration, and eschar formation while IM delivery was associated with higher rates of injection site pain, bruising, and muscle soreness. There were no discernable differences in the frequency or severity of local site reactions vaccine and placebo arms of the study.

Systemic AEs observed during the study were generally mild and transient. Of the 162 systemic adverse events reported, 108 (66%) were judged to be mild (Grade 0 or 1). Fifty one of 162 (31.5%) were of Grade 2 severity and four (2.5%) were of Grade 3 severity. A total of 41 of the 162 (25.3%) systemic adverse events were judged to be at least possibly related to pWRG/VEE or administration procedure. These events included fatigue, headache, low grade fever, dizziness, enlarged lymph nodes, elevated blood pressure, elevated liver enzymes, acute renal insufficiency, and pre-syncope. The only AE of Grade 3 severity that was judged to be at least possibly related to the vaccine candidate was a report of severe fatigue which resolved within one day.

There were three SAEs reported during the study. All of the events, which included occurrences of Grade 3 terminal ileitis, Grade 3 microperforation of the ileum, and Grade 3 small bowel obstruction, occurred in one subject and were associated with the recurrence of previously diagnosed ulcerative colitis. Based on the subject's medical history and the timing of the events, the investigator concluded that they were unrelated to pWRG/VEE or the administration procedure.

There were no discernable differences in the occurrence of systemic AEs between the two routes of administration or between placebo and active arms. Overall, there were no serious or unanticipated safety concerns associated with pWRG/VEE or delivery method identified during the Phase 1 study.

3.4. Tolerability of Vaccine Procedures

To assess the tolerability and acceptability of the administration procedure, all subjects were requested to complete a questionnaire at each dose administration visit.

The questionnaire comprised characterization of the pain perceived by the subject following electrode/needle insertion, during electrical stimulation, and then at 10 and 30 minutes after the administration procedure using a visual analog pain scale (VAS) scored from 0 - 10. In addition, the subjects were asked to provide their opinion regarding the acceptability of the procedure to improve either the prevention or treatment of disease.

For both routes of administration, VAS pain scores peaked at the time of electrical stimulation and decreased significantly at 10 and 30 minutes post administration. The mean pain score reported by individuals receiving the VEEV DNA vaccine by ID-EP was 2.9 (95% CI: 2.3 – 3.5) at the time of electrical stimulation. The mean pain scores for these subjects at 10 and 30 minutes post administration were 0.4 (95% CI: 0.2 – 0.6) and 0.3 (95% CI: 0.1 – 0.5) respectively, which were both significantly decreased relative to the score reported at electrical stimulation ($p < 0.01$, Student's t test) . The mean pain score reported for the subjects receiving the VEEV DNA vaccine by IM-EP was 4.9 (95% CI: 4.2 - 5.7) at the time of electrical stimulation. The mean pain scores for these subjects at 10 and 30 minutes post administration were 2.9 (95% CI: 2.3 – 3.5) and 2.5 (95% CI: 1.9 -3.1) respectively which were both significantly decreased relative to the score reported at electrical stimulation ($p < 0.01$, Student's t test). The difference in mean pain scores between the two delivery methods was also statistically significant ($p < 0.01$, Student's t test). As a subjective measure of tolerability, subjects were queried as to whether the EP procedure that they experienced would be acceptable if it protected against a serious disease for which no other vaccine currently was available. A substantial majority of subjects indicated that both the ID-EP

and IM-EP procedures would be acceptable (Fig. 2). Collectively, these data indicate that while the subjects receiving the ID-EP procedure reported lower pain scores, both procedures were considered to be generally acceptability for use in the prophylactic setting.

3.5. Vaccine immunogenicity

A secondary objective of the study was to assess the frequency, magnitude, and kinetics of VEEV-specific immunological responses elicited by pWRG/VEE delivered by IM- or ID-EP. Serum samples were collected at seven time points during the study. These included pre-screening and then study days 14, 42, 70, 140, 224 and 365. VEEV-neutralizing antibodies in sera were measured by PRNT. The immunogenicity endpoints for each study group were defined as: (i) the frequency of seroconversion over the course of the study; ii) the geometric mean $PRNT_{80}$ titer at each time point; iii) the mean time to seroconversion; and, iv) the frequency of anti-VEEV response at study completion.

VEEV-neutralizing antibodies were not detected in any of the subjects after the first vaccination. After two doses, all subjects in the high dose IM-EP cohort, and five of eight subjects in the low dose IM-EP cohort and in the high dose ID-EP group had measureable neutralizing antibodies by PRNT **(Table 3).** All remaining subjects in the low dose IM-EP group and five of eight subjects in the low dose ID-EP group had developed neutralizing antibodies to VEEV after the third vaccination (**Table 3**). VEEV-neutralizing antibodies were also measured in blood samples collected at the final visit of each subject (day 360). All but one subject in the high dose IM-EP group maintained

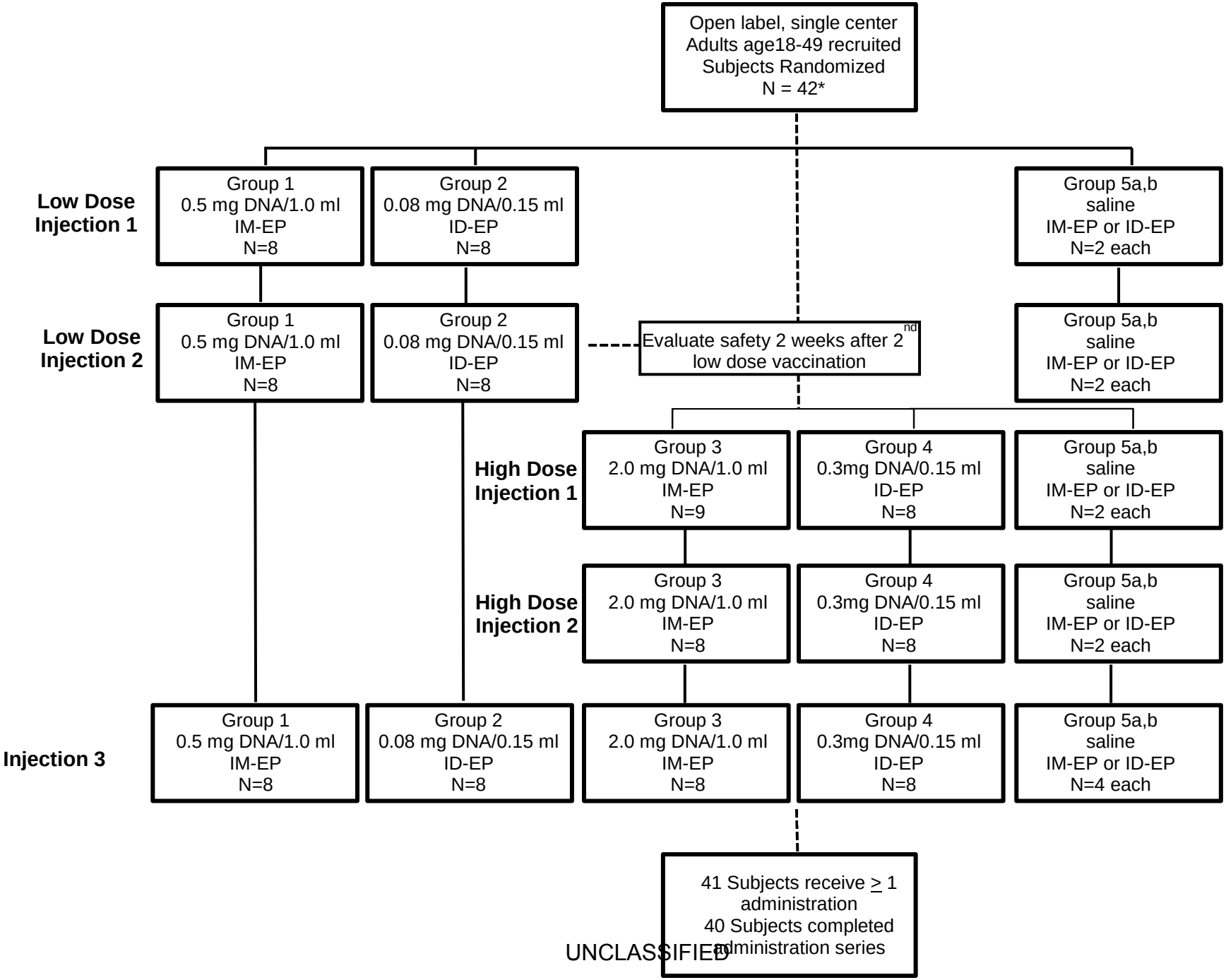
measureable neutralizing antibodies to VEEV (Table 3). The mean time to seroconversion was approximately the same for both IM-EP groups and the high dose ID-EP group (42-43.5 days), whereas the low dose ID-EP group had a longer time to seroconversion (70 days) (**Table 3**).

The kinetics of the neutralizing antibody response as represented by the geometric mean $PRNT_{80}$ titer show that although a majority of subjects in all groups met the criteria for anti-VEEV seroconversion, there was a clear dose effect on the time to seroconversion for both routes of administration tested. Peak titers for all experimental groups were observed at study day 70, with the highest and fastest neutralizing antibody response observed in the high dose IM-EP group (Figure 3). Interestingly, although the high dose ID-EP group received less DNA than the low dose IM-EP group, the kinetics and magnitude of the antibody responses for these cohorts were nearly the same (**Fig. 3**).

Conclusions

The results of the Phase I clinical study of EP-mediated pWRG/VEE delivery demonstrate an acceptable safety and reactogenicity profile for the DNA vaccine candidate when administered by ID- or IM-EP with the TriGrid Delivery System. While there were subtle differences in the nature of the reactogenicity events observed in the IM- and ID-EP groups, the overall frequency and severity of injection site reactions were comparable between the two routes of administration. A DNA dose dependent relationship between seroconversion frequency, time to seroconversion, and magnitude of VEEV-neutralizing antibody responses were observed for both the IM and ID routes

of administration. Although the highest seroconversion frequency, time to seroconversion, and magnitude of anti-VEEV immune response were observed in the IM-EP groups, it is not possible to discern whether this is due to intrinsic properties of the IM route or due to the higher absolute pWRG/VEE DNA doses administered via the IM route in this study. Assessment of the tolerability and acceptability of the administration procedure indicated higher VAS pain scores associated with the IM-EP groups as compared to the ID-EP groups. However, interestingly, there were no significant differences in the subjects rating of the overall acceptability of these two administration procedures for use in prophylactic vaccine delivery for serious infectious diseases. Combined with the promising outcome of non-clinical studies, the safety, tolerability, and immunogenicity assessments conducted during this Phase 1 study indicate that further clinical development of the pWRG/VEE candidate is warranted. While the available data indicate that the evaluation of the IM-EP route should be prioritized, testing of ID-EP delivery at higher DNA concentrations may also be of benefit.


Open label, single center
Adults age18-49 recruited
Subjects Randomized
N = 42*
Low Dose Injection 1
Group 1
0.5 mg DNA/1.0 ml
IM-EP
N=8
Group 2
0.08 mg DNA/0.15 ml
ID-EP
N=8
Group 5a,b
saline
IM-EP or ID-EP
N=2 each
Low Dose Injection 2
Group 1
0.5 mg DNA/1.0 ml
IM-EP
N=8
Group 2
0.08 mg DNA/0.15 ml
ID-EP
N=8
Evaluate safety 2 weeks after 2nd low dose vaccination
Group 5a,b
saline
IM-EP or ID-EP
N=2 each
High Dose Injection 1
Group 3
2.0 mg DNA/1.0 ml
IM-EP
N=9
Group 4
0.3mg DNA/0.15 ml
ID-EP
N=8
Group 5a,b
saline
IM-EP or ID-EP
N=2 each
High Dose Injection 2
Group 3
2.0 mg DNA/1.0 ml
IM-EP
N=8
Group 4
0.3mg DNA/0.15 ml
ID-EP
N=8
Group 5a,b
saline
IM-EP or ID-EP
N=2 each
Injection 3
Group 1
0.5 mg DNA/1.0 ml
IM-EP
N=8
Group 2
0.08 mg DNA/0.15 ml
ID-EP
N=8
Group 3
2.0 mg DNA/1.0 ml
IM-EP
N=8
Group 4
0.3mg DNA/0.15 ml
ID-EP
N=8
Group 5a,b
saline
IM-EP or ID-EP
N=4 each
41 Subjects receive ≥ 1 administration
40 Subjects completed administration series

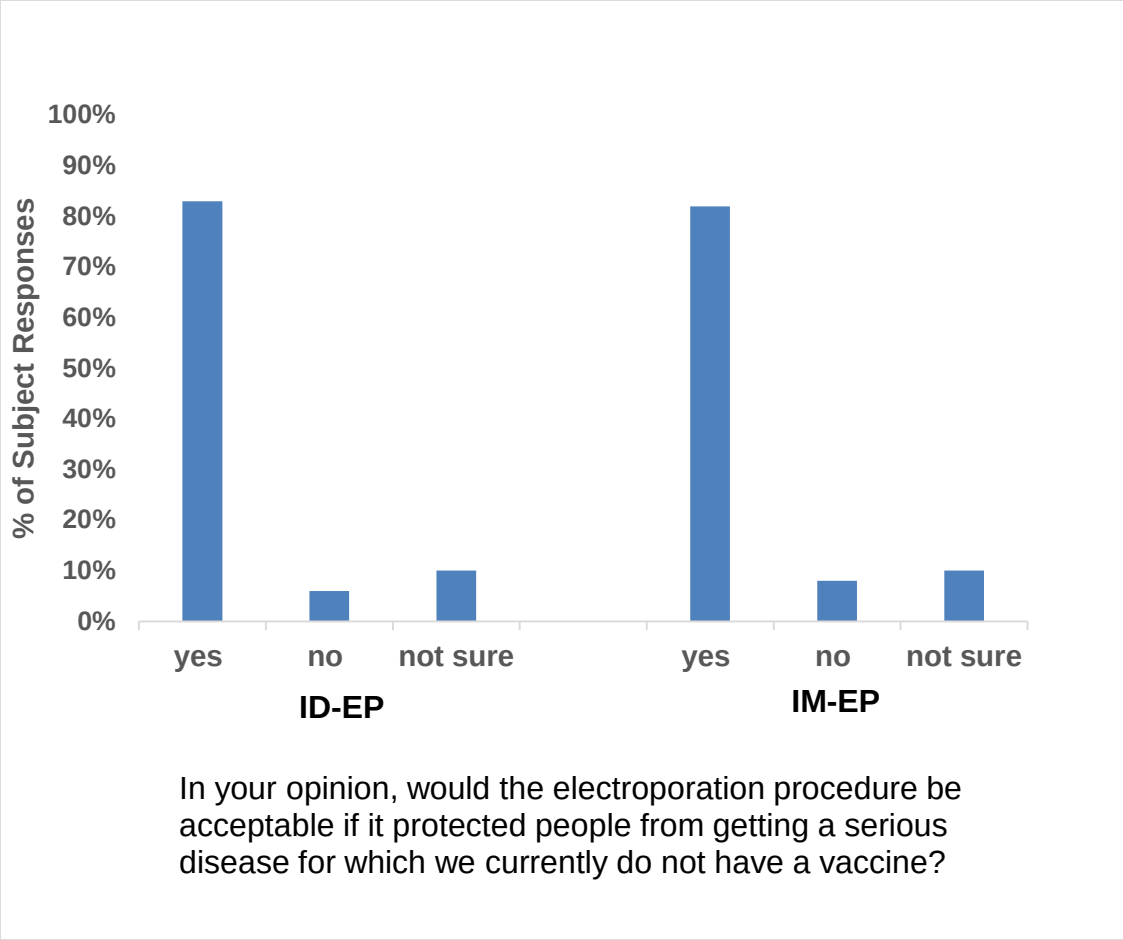


Fig. 2. Acceptability of EP administration procedures. Study participants randomized to receive pWRG/VEE or placebo via IM-EP or ID-EP completed a questionnaire to assess the acceptability of the procedure for potential application in vaccine delivery. Results represent a total of 121 responses from 41 study participants.

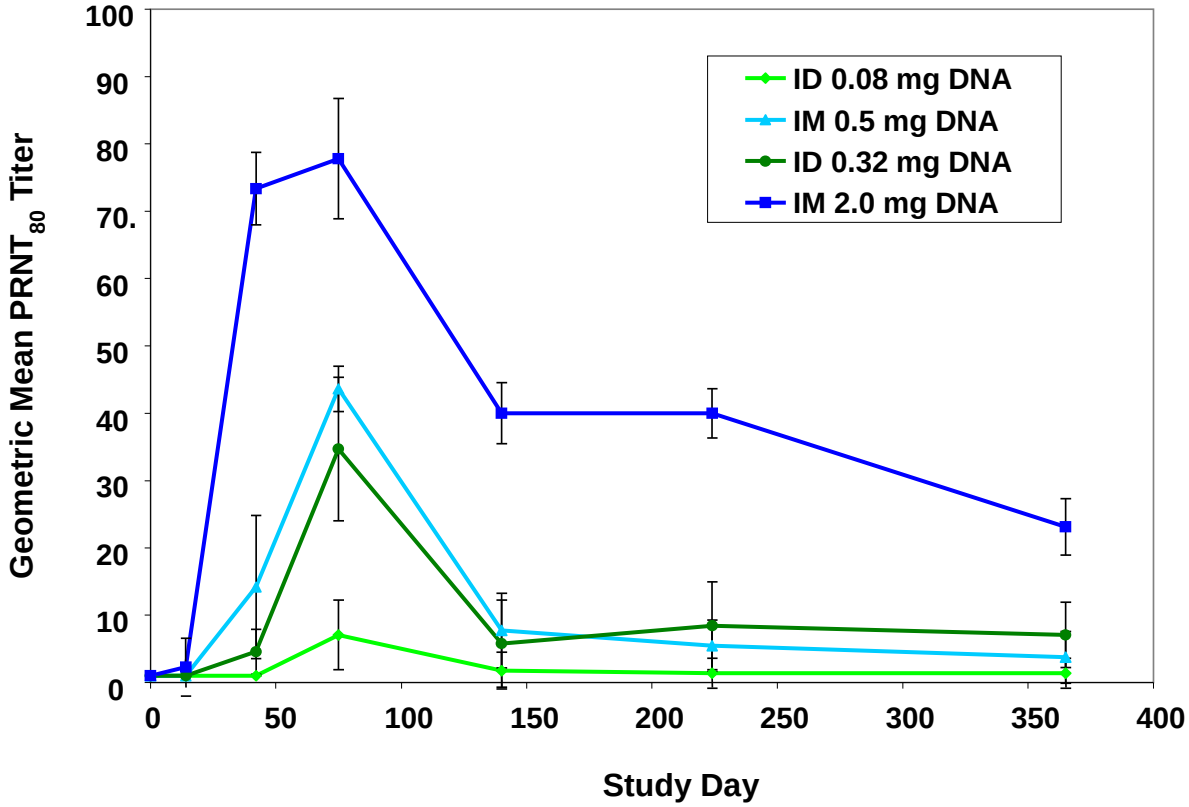


Fig. 3. VEEV-neutralizing antibody responses. Neutralizing antibodies against VEEV IAB (strain Trinidad donkey), were measured by PRNT using serum samples collected from subjects at times shown. Neutralizing antibody titers were calculated as a reciprocal of the highest dilution resulting in an 80% reduction of the plaque number as compared to virus-only control wells ($PRNT_{80}$). Geometric mean titers (GMT) for each of the groups are shown for each of the assay dates.

Figure Legends

Fig. 1. A total of 42 subjects aged 18 through 47 who had no prior history of VEEV exposure or vaccination and exhibited no detectable anti-VEEV response by PRNT prescreening were enrolled in this study. The study population, pre-defined as the number of subjects administered at least one dose of the vaccine candidate or placebo, comprised a total of 41 subjects. The subjects were randomized to receive either three doses of pWRG/VEE or three doses of placebo (0.9% sodium chloride for injection (Hospira, Inc. Lake Forest, IL) at study days 0, 28, and 56 administered by either ID-EP or IM-EP. Subjects were then followed for ten months after the third administration. Following consent and successful screening, each subject was randomized to receive either pWRG/VEE or placebo by ID-EP or IM-EP using a pre-determined sequence of randomization numbers according to the randomization code. Placebo controls were enrolled in parallel with pWRG/VEE arms at a ratio of 1:4. Once all screening procedures were completed and study eligibility was confirmed, the randomization numbers of the pre-determined sequence were allocated sequentially to subjects within the appropriate study arm. Subjects were enrolled in two cohorts corresponding to the two pWRG/VEE concentrations administered to subjects randomized into the active arms of the study (0.5 mg/ml (low dose) or 2.0 mg/ml (high dose). Injection volumes were 150 µl for ID-EP or 1000 µl for IM-EP. Final pWRG/VEE doses were 0.5 or 2.0 mg of DNA delivered by IM-EP and 0.08 or 0.3 mg delivered by ID-EP. Once all subjects in the low dose cohort completed the two week follow up visit after the second administration, a safety

review was conducted by the medical monitor before proceeding with the enrollment to the high dose cohort.

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Table 1: Subject Demographics

Subjects	Number	%
Screened	45	
Randomized	42	
Treated (Safety Population)	41	97.6
Completed Study Procedures	38	90.5
Withdrawn	4[a]	9.5
Gender		
Male	16	39
Female	25	61
Total	41	100
Race		
American Indian or Native Alaskan	0	0
Asian	2	4.9
Black or African-American	10	24.4
Native Hawaiian or Other Pacific Islander	2	4.9
White	20	48.8
Other	7	17.1
Total	41	100
Ethnicity		
Hispanic/Latino	13	31.7
Not Hispanic/Latino	28	68.3
Total	41	100

[a]Two subjects moved from the study area and two subjects withdrew consent not due to AE

Table 2. Adverse Events[a] by Treatment Group

Parameter	Placebo (N=8)	IM-EP 0.5mg/ml (N=8)	ID-EP 0.5 mg/ml (N=8)	IM-EP 2.0 mg/ml (N=9)	ID-EP 2.0 mg/ml (N=8)	Total (N=41)
Subjects with at Least One AE	8	8	8	9	8	41
At least 1 Vaccine Related AE	8	7	8	9	5	37
At least 1 Procedure Related AE	8	8	8	9	8	41
At least 1 Serious AE	0	0	1	0	0	1
At least 1 AE with Outcome of Death	0	0	0	0	0	0
At least 1 AE Leading Withdrawal	0	0	0	0	0	0
Subjects with at Least 1 AE by Severity[a]						
Mild (Grade 1)	3	3	2	1	4	13
Moderate (Grade 2)	5	5	4	6	4	24
Severe (Grade 3)	0	0	2	2	0	4
Potentially Life Threatening (Grade 4)	0	0	0	0	0	0

[a]A subject was counted once in the most severe category if the subject reported one or more events, but different severity. Unknown severity is treated as severe.

Table 3: Seroconversion by treatment group

Group #	Subjects /Group	Dose (mg DNA)	EP Delivery	Number of Subjects Seroconverting (%)		Mean Days to Seroconversion	Day 70 $PRNT_{80}$ GMT (Range)	Day 70 $PRNT_{50}$ GMT (Range)	Subjects Seropositive at Day 365 (%)
				2 doses	3 doses				
1	8	0.08	ID	0 (0%)	5 (62.5%)	70.0	7 (0-80)	39 (0-320)	1 (12.5%)
2	8	0.5	IM	5 (62.5%)	8 (100%)	42.0	44 (10-640)	174 (40-2560)	4 (50.0%)
3	8	0.3	ID	5 (62.5%)	7 (87.5%)	43.5	35 (0-1280)	156 (0-10240)	5 (62.5%)
4	9	2.0	IM	9 (100%)	9 (100%)	42.0	78 (10-1280)	698 (160-20480)	8 (88.9%)
5a	4	-	ID	0 (0%)	0 (0%)	NA	NA	NA	NA
5b	4	-	IM	0 (0%)	0 (0%)	NA	NA	NA	NA
Total	40								

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Conflict of Interest Statement: Drew Hannaman is Vice President, Research and Development of Ichor Medical Systems, Inc. Lesley Dupuy and Connie Schmaljohn

have a patent application pending on the pWRG/VEE DNA vaccine tested herein. The funding agency had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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